

Claims

1. A method for the manufacture of a library of complexes comprising templated molecules, said method comprises the steps of
- a) providing a plurality of different templates comprising a number of coding regions and a reactive group, wherein each coding region of a specific template specifies a unique codon,
 - b) providing a plurality of different building blocks, each building block comprising an anti-codon, a functional entity and a linker connecting the anti-codon and the functional entity, wherein the anti-codon of each building block complements a unique codon of a template, and the functional entity comprises at least one reactive group,
 - c) contacting the plurality of different templates with a subset of the plurality of different building blocks, said subset having anti-codons which complement the unique codons of a specific coding region, said contacting being performed under conditions which allow specific hybridisation of the anti-codons to the unique codons of the templates,
 - d) reacting the reactive group of the template and the reactive group of the building block to obtaining a chemical connection,
 - e) contacting under conditions allowing specific hybridisation, the plurality of different templates harbouring the nascent templated molecules with a further subset of the plurality of building blocks, said subset having anti-codons complementary to the unique codons of a coding region in the vicinity of the coding region harbouring the nascent templated molecules,
 - f) allowing the functional entities of the subset of further building blocks to form a chemical connection to the nascent templated molecules,
 - g) optionally, cleaving one or more of the linkers, provided that at least one linker remains to connect the nascent templated molecule with the template which directed the synthesis thereof,
 - h) optionally repeating steps e) through g),
 - i) obtaining a templated molecule attached via the linker one or more building blocks to the template which directed the synthesis thereof.

2. The method according to claim 1, wherein the reactive group of step a) comprised by the template is covalently attached to the template.

5 3. The method according to claim 1, wherein the reactive group of the template is non-covalently attached to the template.

10 4. The method according to claim 3, wherein the reactive group of the template is covalently attached to a complementing element hybridised to the template.

5. The method according to claim 3 or 4, wherein the reactive group of the template is part of a building block.

15 6. The method according to any of the claims 3 to 5, wherein the building blocks harbouring the reactive group of step a) and the subset of building blocks contacted with the templates in step c) are positioned next to each other.

20 7. The method according to any of the preceding claims, wherein the individual coding regions of the plurality of templates are positioned next to each other in a linear sequence.

25 8. The method according to claims 1 to 6, wherein the template is branched.

9. The method according to any of the claims 1 to 8, wherein coding regions are separated by a spacer group.

30 10. The method according to claim 9, wherein the spacer group identifies the neighbouring coding region or unique codon.

11. The method according to any of the preceding claims, wherein the number of coding regions is 3 to 100.

5 12. The method according to any of the preceding claims, wherein the number of unique codons within a coding region is between 1 and 10,000.

13. The method according to any of the preceding claims, wherein each unique codon is a sequence of 3 to 100 nucleic acid monomers.

10 14. The method according to claim 13, wherein each unique codon comprises a sequence of 8 to 30 nucleic acid monomers.

15 15. The method according to any of the preceding claims, wherein the individual unique codon:anti-codon hybrids within a specific coding region have a similar annealing temperature.

16. The method according to any of the claims 1 to 14, wherein the individual unique codon:anti-codon hybrids within a specific coding region have a different annealing temperature.

20 17. The method according to any of the preceding claims, wherein the functional entity of a building block comprises a reactive group capable of forming a connection to a reaction partner of another functional entity or nascent templated molecule.

25 18. The method according to any of the preceding claims, wherein the functional entity of a building block comprises a reactive group capable of forming a connection to a reactive group of another functional entity or nascent templated molecule through a bridging fill-in group.

30 19. The method according to any of the preceding claims, wherein the linker is attached to the anti-codon oligonucleotide at a central area thereof.

20. The method according to any of the preceding claims, wherein the anti-codon and the linker is a contiguous linear oligonucleotide.

5 21. The method according to any of the preceding claims, wherein the linker is attached to the functional entity through a reactive group capable of forming a connection to another functional entity or a nascent templated molecule.

10 22. The method according to claim 21, wherein the linker is capable of being cleaved simultaneously with the formation of the connection.

15 23. The method according to any of the preceding claims, wherein the reactive groups involved in the formation of the connection between functional entities or a functional entity and a nascent templated molecule are reactions partners.

20 24. The method according to any of the preceding claims, wherein the subset in steps c) comprises building blocks having anti-codons which form hybrids with unique codons in a coding region neighbouring the reactive group of the template.

25 25. The method according to any of the preceding claims, wherein the subset in step e) comprises building blocks having anti-codons which form hybrids with unique codons in a coding region neighbouring the building block harbouring the nascent templated compound.

26. The method according to claims 24 or 25, wherein the subset is formed by adding the building blocks separately.

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27. The method according to any of the claims 24 or 25, wherein the sub-sets in steps c) or e) are formed by directing the annealing temperature of the individual building blocks.

5 28. The method according to any of the preceding claims, wherein the anticodon of a building block with a functional entity is ligated to the anti-codon of a building block harbouring a nascent molecule prior to establishing the connection between the functional entity and the nascent molecule being prepared.

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29. The method according to any of the preceding claims, wherein building blocks intended to interact with each other each are provided with a part of a molecule pair being capable of reversible interaction.

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30. The method according to claim 29, wherein the one part of the molecule pair is present on the linker, close to the functional entity or nascent templated molecule.

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31. The method according to claims 29 or 30, wherein the one part of the reversible interacting molecule pair of a first building block is an oligonucleotide and the other part of the reversible interacting molecule pair of a second building block intended to interact with the first building block is a complementing oligonucleotide.

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32. The method according to the preceding claims 29 to 31, wherein the annealing temperature of an interacting molecule pair is lower than the annealing temperatures for the unique codon:anti-codon hybrids of the involved building blocks.

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33. The method of claim 32, wherein the annealing temperature of the reversible interacting molecule pair is below room temperature but above 5°C.

34. The method according to claim 33, wherein the annealing temperature is between 10°C and 20°C.

5 35. The method according to any of the preceding claims, wherein the linker is rigid and attached the anti-codon through a molecular hinge.

36. The method according to claim 35, wherein the rigid linker is a double stranded oligonucleotide.

10 37. The method according to claim 35 or 36, wherein the molecular hinge is a single stranded region of the building block.

38. The method for the manufacture of a library according to claim 1, wherein the complexes obtained comprise templated molecules attached to the template which templated the syntheses thereof via a single building block.

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39. The method according to claim 1, comprising the further step of connecting the templated molecule with the template which directed the syntheses thereof, or a complementing template, via a covalent link.

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40. The method according to claim 39, wherein the covalent link is selectively cleavable to provide for a release of the templated molecule.

25 41. The method according to claim 1, wherein the templated molecules of the library complex are polymers.

42. The method according to any of the claims 1 to 41, wherein the optional cleavage of some or all of the linkers of step g) are not performed.

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43. The method according to claim 42, comprising the further step of cleaving all but one linker after the formation of the templated molecule.

44. The method according to any of the preceding claims, wherein the anti-codons following the cleavage of the linker attached thereto, remain hybridised to the unique codons.

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45. The method according to claim 44, wherein the anti-codons attached to the templates are ligated together to create a complementary template.

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46. The method according to claim 1, comprising the further step of transferring the templated molecule to an anchorage point on the template, or a sequence complementing the template, to establish an effective chemical connection.

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47. The method according to claim 46, wherein the complementing sequence has a higher annealing temperature than one or more of the building blocks.

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48. The method according to claim 1, comprising the further step of connecting the templated molecule with a complementary template via a covalent link.

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49. The method according to claim 48, wherein the template is covalently connected to the complementing template.

50. The method according to claim 48, wherein the covalent link is selectively cleavable to provide for a separation of the templated molecule from the complementary template.

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51. A library of complexes obtainable according to any of the claims 1 to 45.

52. A method of enriching a library of complexes comprising templated molecules with respect to a predetermined activity, said enrichment method comprising the steps of:

- i) establishing a first library of complexes comprising templated molecules, said library being obtainable according to any of the claims 1 to 50,
- ii) exposing the library to conditions enriching the library with complexes having the predetermined activity,
- iii) amplifying the complexes of the enriched library,
- iv) optionally, repeating step ii) to iii), and
- v) obtaining an enriched library having a higher ratio of complexes comprising templated molecules with the predetermined activity.

53. The method of claim 52, wherein step iii) comprises a 10^1 to 10^{15} -fold amplification.

54. The method of claim 52, wherein the steps ii) and iii) are repeated at least 2, 3, 5 times, such as at least 10 times, such as at least 15 times.

55. The method of claim 52, further comprising a step of identification of the complexes having the predetermined activity.

56. The method of claim 52, wherein the identification is conducted by analysing the template and/or complementary template associated with the molecule.

57. The method of claim 52, wherein the conditions enriching the library comprises contacting a binding partner to the templated molecules of interest.

58. The method according to claim 57, wherein the binding partner being directly or indirectly immobilised on a support.

59. The method according to claim 52, wherein the enrichment is conducted by screening for complexes having an affinity for – or an effect on – a target molecule or a target entity.

60. The method according to claim 52, wherein the enrichment is conducted by selection for catalytic activity.

61. The method of claim 52, wherein the conditions enriching the library involves any one or more of electrophoretic separation, gelfiltration, immunoprecipitation, isoelectric focusing, centrifugation, and immobilization.

62. The method of claim 52, wherein the conditions enriching the library comprises providing cells capable of internalising the templated molecule, or performing an interaction with the templated molecule having the desired predetermined activity.

63. The method according to claim 52, wherein the amplification of the complexes of the enriched library comprises the steps of

- A. contacting the library of complexes with amplification means,
- B. amplifying the templates or the complementing templates, and
- C. conducting the method according to any of the claims 1 to 50 using the amplification product of step B as templates.

64. A method for the manufacture of a complex of a templated molecule attached to the template which directed the synthesis thereof, said method comprises the steps of

- a) providing a template comprising a number of coding regions and a reactive group, wherein each coding region specifies a unique codon,
- b) providing a plurality of different building blocks, each building block comprising an anti-codon, a functional entity and a linker connecting the anti-codon and the functional entity, wherein the anti-codon of

each building block complements a unique codon of the template, and the functional entity comprises at least one reactive group,

- 5 c) contacting the template with a building block having an anti-codon which complements the unique codon of a specific coding region, said contacting being performed under conditions which allow specific hybridisation of the anti-codon to the unique codon of the templates,
- d) reacting the reactive group of the template and the reactive group of the building block to obtaining a chemical connection,
- 10 e) contacting under conditions allowing specific hybridisation, the template harbouring the nascent templated molecule with a further building block having an anti-codon complementary to the unique codon of a coding region in the vicinity of the coding region harbouring the nascent templated molecule,
- 15 f) allowing the functional entity of the further building block to form a chemical connection to the nascent templated molecule,
- g) optionally, cleaving one or more of the linkers, provided that at least one linker remains to connect the nascent templated molecule with the template which directed the synthesis thereof,
- h) optionally repeating steps e) through g),
- 20 i) obtaining a templated molecule attached via the linker of one or more building blocks to the template which directed the synthesis thereof.

60. A method for preparing a templated molecule, comprising the further step of claim 59 of cleaving the linker(s) of the one or more building blocks to
25 release the templated molecule.